RISK FACTORS FOR THE DEVELOPMENT AND PROGRESSION OF DIABETIC NEPHROPATHY

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ABSTRAK

Factor-faktor risiko yang menyebabkan terjadinya nefropathi diabetes dan menyumbang kepada kemerosotannya.

RINGKASAN

Satu kajian telah dijalankan di Hospital Universiti Sains Malaysia dari tahun 1997 hingga 2000 untuk mengkaji factor-faktor risiko terhadap terjadinya nefropathi diabetes dan menyumbang kepada kemerosotannya di kalangan pesakit diabetes.

OBJEKTIF KAJIAN

1. Untuk mengenalpasti factor-faktor risiko yang menyebabkan terjadinya penyakit nefropathi diabetes
2. Untuk mengenalpasti factor-faktor risiko yang menyumbang kepada kemerosotan nefropati diabetes.

KAEDAH PENYELIDIKAN

Kajian ini dijalankan secara "retrospective" di mana rekod pesakit-pesakit diabetes mellitus jenis 2 yang secara klinikalnya mengalami nefropati atau

tidak. Pesakit-pesakit ini dibahagikan kepada dua kumpulan iaitu normoalbuminuria dan makroalbuminuria berdasarkan kepada tahap kandungan protein dalam urin 24 jam dan juga dalam ujian dipstick urin protein. Data-data yang berkaitan juga diambil dari rekod pesakit.

KEPUTUSAN

Sebanyak 200 rekod pesakit telah dikaji, di mana hanya 86 rekod sahaja yang telah dimasukkan dalam analisis akhir. Daripada jumlah ini, sebanyak 25 pesakit termasuk dalam kumpulan normoalbuminuria dan 61 dalam kumpulan makroalbuminuria. Didapati bahawa hanya darah tinggi sahaja yang merupakan satu-satunya variabel yang mempunyai perbezaan yang signifikan di antara kedua-dua kumpulan tersebut ($p=0.03$). Apabila ujian multivariate menggunakan 'logistic regression' dilakukan, didapati bahawa jangkamasa diabetes, paras trigliserid dan tabiat merokok memberikan sumbangan yang signifikan kepada risiko terjadinya nefropathi diabetes.

Pesakit yang mengalami kemerosotan fungsi buah pinggang yang cepat didapati mempunyai min tekanan darah arteri ($p=0.02$), tekanan darah sistolik ($p=0.03$), tekanan darah diastolik ($p=0.04$) dan juga kandungan

kumuhan protin dalam urin (p=0.01) yang lebih tinggi dan signifikan bila dibandingkan dengan pesakit yang mengalami kemerosotan yang lambat.

Kesimpulan

Faktor-faktor risiko yang di dapati menyebabkan terjadinya nefropati diabetes adalah jangkamasa diabetes, paras trigliserid dan tabiat merokok manakala yang menyumbang kepada kemerosotannya adalah darah tinggi, kandungan protin dalam urin yang dikumpulkan dalam 24 jam dan kehadiran riwayat diabetes dalam keluarga.

Kata kunci

Diabetes mellitus jenis 2, nefropati, faktor-faktor risiko

ABSTRACT

Risk factors for the development and progression of diabetic nephropathy

Introduction

A study had been carried out in Hospital University Sains Malaysia from 1997 to 2000 to investigate on development and progression of diabetic nephropathy.

Objective

1. To identify risk factors for the development of diabetic nephropathy
2. To identify risk factors for the progression of diabetic nephropathy

Methodology

This is a retrospective study. Case records belonging to patients with type 2 diabetes with and without nephropathy were reviewed. They were then

grouped into either normoalbuminuria and macroalbuminuria based on their 24 hour urine protein and urine dipstick test for protein. Other related data was also extracted.

Result

Of the 200 case notes reviewed, only 86 were included in the final analyses. Twenty five of whom had normoalbuminuria and 61 had macroalbuminuria. On univariate analysis, it was noted that hypertension was the only variable showing significant difference between the two groups (P = 0.03). On mutivariate analysis using logistic regression it was noted that the duration of diabetes mellitus, the levels of serum triglycerides and smoking significantly contributed to the development of diabetic nephropathy.

When compared between patients with rapid and slow progression of renal disease, patients with rapid progression had significantly higher mean arterial pressure (P=0.02), systolic blood pressure (P=0.03), diastolic blood pressure (P= 0.04) and has greater protein excretion. In addition the patient with rapid progression had significant strong family history of diabetes mellitus (P = 0.05).

Conclusion

Risk factors for the development of diabetic nephropathy were duration of diabetes, serum triglycerides, and smoking habit while risk factors for the progression of diabetic nephropathy were hypertension, 24 hour urine protein and family history of diabetes.

Keywords

Type 2 diabetes mellitus, nephropathy, risk factors

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LIST OF ABBREVIATIONS

ACE-I	=	Angiotensin converting enzyme inhibitor
AGE	=	Advanced Glycosylation end product
BMI	=	Body mass index
ESRD	=	End stage renal disease
BP	=	Blood Pressure
DN	=	Diabetic Nephropathy
GFR	=	Glomerular filtration rate
JNC	=	Joint National Committee
MAP	=	Mean arterial pressure
MOH	=	Ministry of Health
RCTs	=	Randomised controlled trials
RAS	=	Renin Angiotensin System

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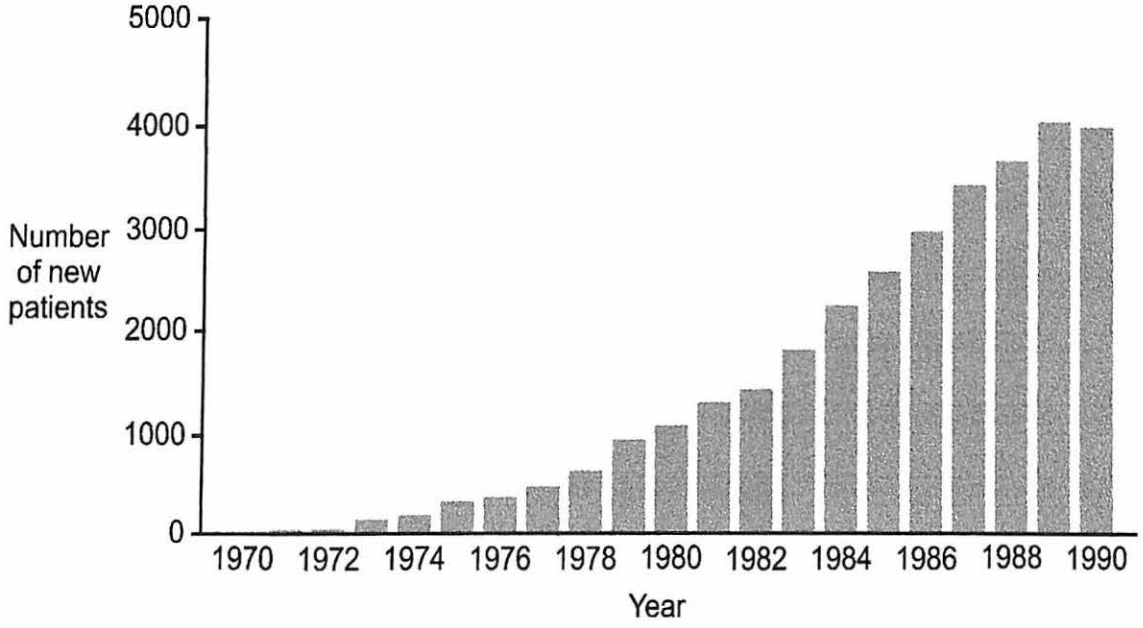
INTRODUCTION

Diabetic nephropathy is a devastating complication of diabetes. Why some diabetic persons are susceptible to diabetic nephropathy and others are not has intrigued investigators. Many believe that hypertension (Hostetter 1982; Schmitz et al. 1994; Parving et al. 1983) and poor glycaemic control lead to nephropathy (RG Larkin 1996). It is likely that the pathophysiology of diabetic nephropathy involves an interaction of metabolic and haemodynamic factors (Cooper at al. 1996). Diabetic nephropathy has become the leading cause (25% to 42% of patients) of end stage renal disease (ESRD) in Europe (fig 1.1), Japan, and the United States.A Similar pattern was seen in Singapore where diabetic nephropathy accounted for 31% of ESRD (Woo 1997). This problem will continue to rise as the incidence of diabetes mellitus throughout the world is expected to double within the next 10 years (fig 1.2).



Adapted from Raine AEG. *Diabetologia* 1993;36:1099-1104

Figure 1.1: Bar chart showing incidence of ESRD due to diabetes in Europe from 1970-90

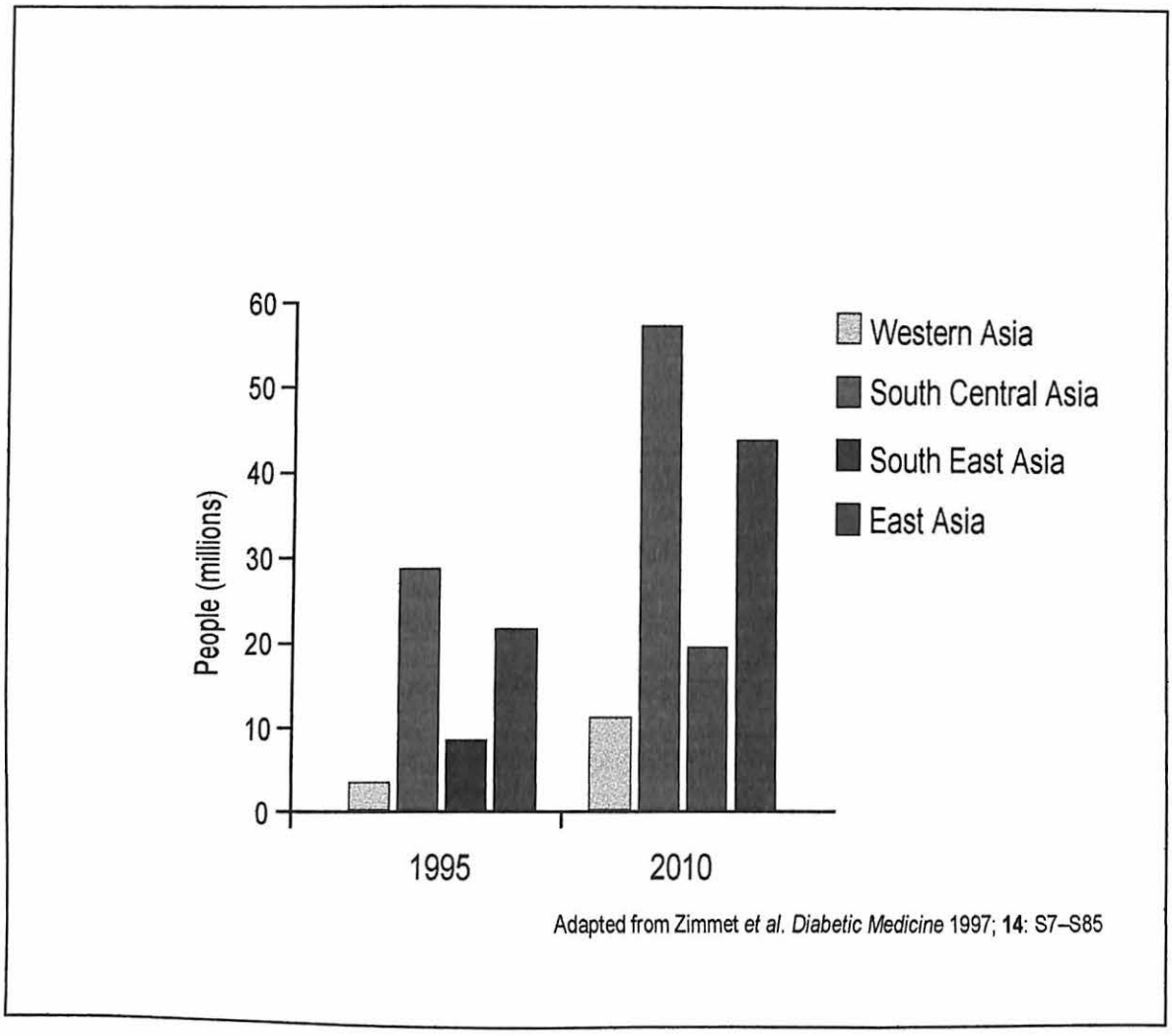


Figure 1.2: Projected rise of diabetes in the world population by 2010

1.1 Epidemiology of diabetic nephropathy

Diabetic nephropathy can occur in both type 1 and type 2 diabetes mellitus, and the risk of nephropathy with progression to end stage renal disease is similar in both groups (Ritz et al. 1999). In a study by Pugh (1993), hyperglycaemia was found to be the most dominant contributing factor for the progression towards ESRD in type 1 diabetes whereas hypertension played a more dominant role in type 2 diabetes. However, the late phase of progressive renal disease towards ESRD in both groups showed similar profile. The epidemiology of diabetic nephropathy in type I diabetes had been well studied as compared to type 2 diabetes. This is because the onset of type I diabetes is obvious and its natural history can be closely followed thereof. In contrast, the date of onset in type 2 diabetes is often unknown and the low grade albuminuria is less specific as an indicator of renal disease progression (Ismail et al. 1999).

The peak onset of nephropathy in Type 1 diabetes is between 10 to 15 years after its onset. For those patients who did not have proteinuria after 20 to 25 years of diabetes, the risk of progressing to overt renal failure was only about one percent per year (Cowie et al. 1989). On the other hand, the Pima Indian has provided useful information on type 2 diabetes. This native

American tribe has the highest prevalence of diabetes in the world where 70% of adults suffer from type 2 diabetes. In this well studied population, the incidence of ESRD attributed to diabetic nephropathy increased from 0 cases/1000 person-years at 0-5 years to 40.8 cases/1000 person-years when the duration of diabetes was more than 20 years (Nelson 1996, 1998).

1.2 Natural history of diabetic nephropathy

Stages of Diabetic Nephropathy according to Mogensen et al (1983) are divided into various phases:

1. Early hypertrophy and hyperfiltration
2. Silent phase
3. Incipient nephropathy
4. Clinical diabetic nephropathy
5. End stage renal disease

The above phases are well described in patients with type 1 diabetes and to some extent in type 2 diabetic patients. Type 2 diabetic patients often suffer from co-existing illnesses such as hypertension and etc. that can also lead to nephropathy via other pathogenetic processes. In a related study, the proportion of type 2 diabetic patients diagnosed to have some primary renal

disease ranged from 23 to 54.3% (Humprey et al. 1989). Therefore the natural history of nephropathy in type 2 diabetic can be unpredictable.

1.2.1 Early Hypertrophy and Hyperfiltration

This phase is characterized by renal enlargement, intrarenal hypertension and high GFR and is seen early in the course of diabetes (Rudberg, 1992). These haemodynamic phenomena are linked with the development of microalbuminuria and are partly reversible by good glycaemic and blood pressure control (Hostetter et al . 1982; Rudberg ,1992).

1.2.2 Silent Phase

During this so-called silent phase early histological abnormalities in the kidney may be seen, including glomerular basement membrane hypertrophy and subtle thickening of glomerular basement membrane which are best seen under electron microscopy (Mogensen 1982).

1.2.3 Incipient Nephropathy

The earliest clinical evidence of nephropathy is the appearance of albumin in urine (>30 but <300mg/day or <20μg/min), referred to as microalbuminuria.(See Table 3) Diabetic patients with microalbuminuria

suffer from incipient nephropathy. A 24-hour urine protein collection is the gold standard for the detection of microalbuminuria (Bojestig et al. 1994). Fever, exercise, heart failure and poor glycaemic control are among the factors that can cause transient microalbuminuria (Mogensen et al. 1981).

Microalbuminuria is associated with raised GFR. A normal GFR will in this context indicate loss of renal function (Damsgaard, 1993). The presence of persistent microalbuminuria is highly significant since it predicts the development of overt renal disease in both type 1 and type 2 diabetes (Viberti, 1982). Furthermore, microalbuminuria is associated with increased risk of cardiovascular and microvascular diabetic complications as well as increased mortality (Dinnen, 1997; Mogensen, 1981), especially in type 2 diabetes. Renal histology at this stage reveals typical glomerulosclerosis.

1.2.4 Clinical Diabetic Nephropathy

Diabetic patients with urinary albumin excretion rates above 20μg/min or 300mg/day qualify for overt diabetic nephropathy. This is usually associated with relentless loss of GFR by (1 to 24 mls/min/year) until ESRF supervenes.

Proteinuria is generally regarded as a marker for the degree of glomerular damage. The level of proteinuria correlates well with the prognosis for renal function. Any intervention that retards the progression of diabetic renal disease also reduces proteinuria (Cooper, 1998)

1.2.5 End Stage Renal Disease

Diabetic patients who require renal replacement therapy to sustain renal function are considered to have ESRD. It is characterized by generalized glomerular death and very low GFR. They usually have GFR of less than 10mls/min.

Table 3. Table showing quantity of urine albumin for different categories of albuminuria in diabetic nephropathy.

Category	24-Hr collection (mg/24h)	Timed collection (µg/min)	Spot collection (µg/mg creatinine)
Normal	< 30	< 20	< 30
Microalbuminuria	30-300	20-200	30-300
Clinical albuminuria	> 300	> 200	> 300

(From American Diabetes Association: Practice Recommendation 2000)

1.3 Risk Factors for development and progression of Diabetes Nephropathy

Diabetes nephropathy is a common problem, most likely to occur in patients who have poor glycaemic control, hypertension, glomerular hyperfiltration, or who are black, Mexican or Pima Indian (Burton, 2001).

1.3.1 Hypertension

Hypertension is much more common amongst diabetic patient than in general population and has been indentified as a major risk factor for both macrovascular and microvascular complications including diabetic nephropathy. Systemic hypertension has adverse effect on the kidney and may initiate the development of renal disease (as in hypertensive nephrosclerosis) or accelerate loss of function in the kidney in which parenchymal disease has already established. The mechanism by which hypertension damages the kidney in renal disease is not clearly understood. It may produce vascular hypertrophy which in turn leads to ischaemic glomerulosclerosis. On the other hand systemic hypertension may induce glomerulosclerosis by producing intraglomerular hypertension, which increases glomerular blood flow and filtration rate per nephron, leading to endothelial-cell damage (Klahr et al. 1998). Many patients with type 2

diabetes are hypertensive from the onset of diabetes as opposed to type 1 diabetes, in whom hypertension develop later (Simonson, 1988).

1.3.2 Glycaemic control

Type 1 and type 2 diabetes both have in common the state of chronic hyperglycaemia, and glucose dependent processes are likely involved in the pathogenesis of diabetic complications, including nephropathy. Glucose-induced tissue injury may be mediated by the generation of advanced glycated proteins or via other mechanism such as poly-ol pathway, both of which have been implicated in nephropathy (Larkin et al. 1992).

1.3.3 Hyperlipidaemia

Hyperlipidaemia is common in both type 1 and type 2 diabetes. Raised plasma TG and low level of HDL has been correlated with the development of diabetic nephropathy as well as with cardiovascular complication (Gilbert et al. 1993; Nyberg et al.1987). Several studies in patient with type 1 and type 2 diabetes have shown correlation between serum cholesterol concentration and progression of diabetic nephropathy (Breyer 1996, Gall 1993, Stefanski 1996). Moorheaed JF (1982), hypothesized that chronic progressive kidney disease may be mediated by abnormal lipid metabolism. A series of self-perpetuating secondary events follow an initial glomerular

injury. Increased glomerular basement membrane permeability leads to loss of lipoprotein lipase activators, resulting in hyperlipidaemia. The close resemblance of mesangial cells to smooth muscle cells and the key role of the latter in the pathogenesis lead to atherosclerosis. Lipoprotein which function as a carrier for lipid, has surface receptors in many cells, including smooth muscle cells and probably mesangial cells. (Saulo Klahr, 1988).

1.3.4 Genetic Susceptibility and Race

Genetic susceptibility may be an important determinant of both the incidence and severity of diabetic nephropathy (Cooper et al. 1998; Krolewski 1999). Krolewski (1999) also showed that the likelihood of developing diabetic nephropathy was markedly increased in patients with diabetic siblings or parents who also had diabetic nephropathy. This observation had been made in both type 1 and type 2 diabetes. (Krolewski, 1999). People are looking at the influence of genes as a cause of diabetic nephropathy. It is unlikely to expect only a single gene to be involved. Multiple genes may contribute because diabetes mellitus is considered as a complex multifactorial disease (Remuzzi et al. 1998).

Can we therefore invoke genetic susceptibility in diabetic nephropathy? Quinn (1996) reported that diabetic siblings of a person with type 1 diabetes

and nephropathy has a 72% cumulative risk of developing renal disease, while a diabetic sibling of a person with type 1 diabetes but without nephropathy only has 25% risk.

One report for example, evaluated a Pima Indian family with 2 generations who had type 2 diabetes. The likelihood of their offspring developing overt proteinuria was 14%, 23% and 46% if neither parents had proteinuria, one parent with proteinuria, and both parents had proteinuria respectively (Pettitt, 1990). Therefore genetic factors are likely to be important in diabetic nephropathy. Recent interest has focused on genes of the Renin Angiotensin System, which are known to be polymorphic and have been extensively studied in relation to cardiovascular disease. Deletion-deletion (DD polymorphism) has been associated with an increased risk for the development of diabetic nephropathy, more severe proteinuria and a greater likelihood of progressive renal failure (Jeffers, 1997).

Other factors that have been implicated in the development of diabetic nephropathy included; Transforming Growth Factor Beta (TGFβ) gene (Sharma et al. 1997) and advanced glycation end (AGE) product (Makita, 1991).

The incidence and severity of DN were found increased in black (3 to 6 fold compared to Caucasians), Mexican-Americans and Pima Indian with type 2 diabetes mellitus.

1.3.5 Smoking

Smoking is an important predictor of risk for renal complication in type 1 diabetes (Sawicki, 1994). Olivarus et al. (1993) examined male patients with newly diagnosed type 2 diabetes and found that heavy albuminuria was more common in smokers (8.2%) and former smokers (7.3%) than in non smokers (2.1%).

Tobacco smoking can cause vasoconstriction, impaired platelet function, and abnormal regulation of coagulation and blood pressure (Patterson, 1998). Smoking was associated with an increase in serum creatinine (Bleyer, 2000).

1.4 Treatment

Treatment for diabetic nephropathy can be divided into:

a. **Primary prevention:** Treatment applied to normoalbuminuria patient

b. **Secondary prevention:** Treatment applied to diabetic patients at high risk (microalbuminuria)

c. **Tertiary prevention:** Treatment of overt nephropathy aimed at preventing or delaying development of ESRD

1.4.1 Primary prevention

Several studies showed that glycaemic control will delay progression to nephropathy in normoalbuminuria (Wang 1993, DCCT trial 1993). Three RCTs documented a benefit in blood pressure control which retards the development of diabetic nephropathy, in type 1 and type 2 diabetes (Euclid study group 1997; Ravid , 1998; Hope study 2000).

1.4.2 Secondary prevention

A meta-analysis of 12 trials in 698 type 1 diabetic patients with microalbuminuria who were followed up for at least one year revealed that

ACE-inhibition reduced the risk of progression to macroalbuminuria by 62% compared to that of the placebo group [odds ratio 0.38 (95% CI, 0.25 to 0.57)] (The ACE inhibitor in Diabetic Nephropathy Trialist Group. 2001). The result in type 2 Diabetes also showed similar beneficial effect when followed up for 15 years (UKPDS 1998 ;Steno Type 2 .1999). It was also shown that good blood pressure control slows down renal function deterioration regardless of the type of antihypertensive used (UKPDS 1998).

1.4.3 Tertiary prevention

Blood pressure lowering in Type 1 Diabetes with nephropathy for long term anti-hypertensive (Mogensen 1982). There is evidence for a beneficial effect of ACE-I above and beyond blood pressure control (Bjorck et al. 1992 ;Lewis et al. 1993)

1.5 RECENT TRIALS using Angiotensin receptor blockade

1.5.1 IRMA II (Irbesartan Microalbuminuria Type 2 Diabetes in Hypertension Patient)

Irbesartan was shown to significantly slow the development of DN. It was also shown to have renoprotective properties independent of its

blood pressure-lowering effect in patient with type 2 diabetes and microalbuminuria. At 2 years, the adjusted risk of developing nephropathy was reduced by 68 % and the albumin excretion rate improved by 46 % in the high dose Irbesartan group (300mg/d) compared with placebo. (Parving et al. 2001).

1.5.2 **IDNT** (Irbesartan Diabetic Nephropathy Trial)

In another study also using Irbesartan it was shown to be effective in protecting against the progression of nephropathy due to type 2 diabetes. (Lewis EJ et al. 2001)

1.5.2 **RENAAL** (Reduction of End points in Non insulin dependant with Angiotensin II Antagonist Losartan)

This was a study of patients on Type 2 Diabetes with protenuria and elevated serum creatinine. They were randomized either to Losartan or Placebo and continued to receive conventional antihypertensive to achieved blood pressure control. The use of Losartan showed a 16 % relative risk reduction in doubling of serum creatinine level. It also showed reduced risk in the development of ESRD or death.

($p = 0.024$)(Brenner et al. 2001)

To conclude, It is likely that potential interplay between metabolic and haemodynamic factors involved in the pathogenesis of DN. This is best illustrated in the following diagram (Cooper 1996).

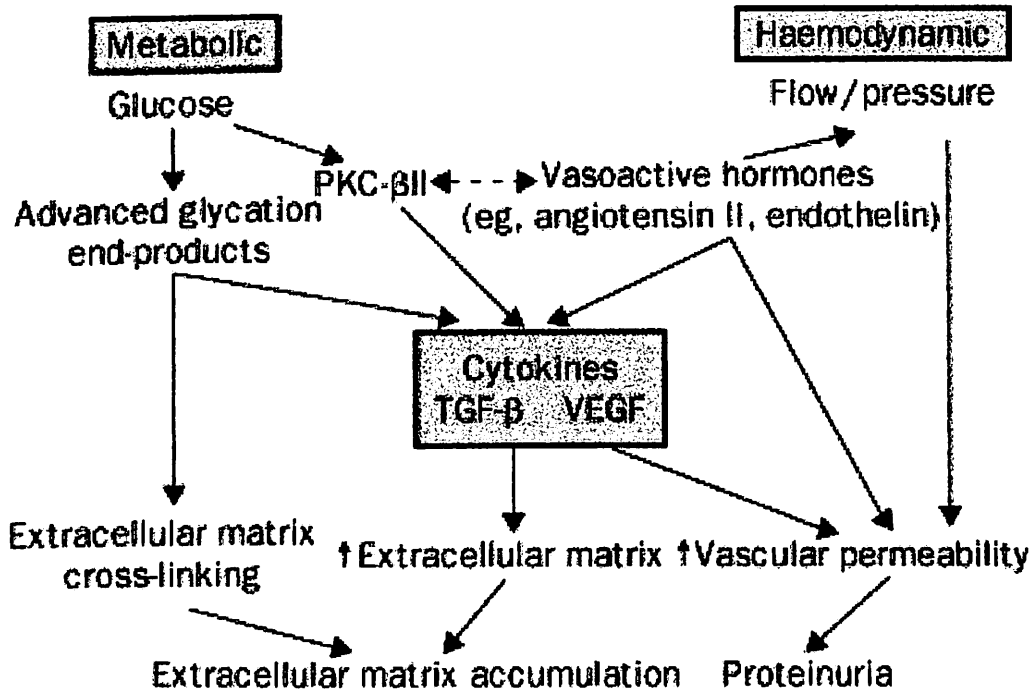


Figure 1.3: Schematic representation of the biochemical and haemodynamic pathophysiology in the renal microcirculation of diabetic nephropathy.

Legends:

PKC:Protein kinase C

TGF-ß:Transforming growth factor- Beta

VEGF:Vascular endothelial growth factors

OBJECTIVES

2.1 Primary:

1. To identify risk factors for the development of diabetic nephropathy
2. To identify risk factors for progression of diabetic nephropathy

2.2 Secondary:

1. To know the prevalence of hypertension in type 2 diabetes mellitus patients
2. To know the prevalence of retinopathy in type 2 diabetes mellitus
3. To know the type of antihypertensive prescribed to the study population

METHODOLOGY

3.1 Research design and methodology

This study is a retrospective study conducted at Hospital University Science Malaysia from 1997 to 2001.

3.2 Subjects

Two hundreds case records were selected from the Endocrine Clinic as well as from the medical wards in Hospital University Sains Malaysia for review. A total of 86 patients who fulfilled the study inclusion criteria and who did not have exclusion criteria were further analysed.

3.3 Inclusion criteria

1. Type 2 Diabetes Mellitus
2. Case records with documented 3 serum creatinine or more done within the last 3 years and the interval between the first and the third serum creatinine should be at least 2 years apart.

3.4 Exclusion criteria

1. DM-Type 1
2. End stage renal failure requiring dialysis
3. Positive microalbuminuria (dip stick test)
4. Presence of renal diseases other than diabetic nephropathy i.e urinary tract infection, renal calculi, glomerulonephritis.
5. Overt heart failure
6. Hemoglobinopathy

The following data was extracted from his/her case records.

1. Age and Gender
2. Smoking habits
3. Family history of diabetes
4. Duration of diabetes
5. Weight (kg)
6. Height (m)
7. Blood pressure
8. Serum creatinine at least 3 times at various interval
9. Level of HbA1c
10. Total cholesterol and triglyseride

11. Dipstick urine albumin
12. 24 hour urine protein over the last 6 months.
13. History of retinopathy
14. History of hypertension
15. Medication (Oral hypoglycemic and antihypertensive)

Creatinine clearance was calculated using Cockcroft formula for adult (Klahr 1991)

1. Adult male:

$$CrCl = \frac{((140-Age) \times (weight) \times 88.33\ [conversion\ factor\ to\ mg/dl])}{72 \times Serum\ Creatinine\ (mmol/L)}$$

2. Adult female:

$$CrCl = \frac{0.85 \times ((140-Age) \times (weight) \times 88.33)}{72 \times Serum\ Creatinine\ (mmol/L)}$$

3.5 Measurements Method.

1. **Plasma glucose**: This was measured by glucose-oxidase method using a Beckman glucose analyzer.

2. **Triglyceride, Total Cholesterol:** The assays were done by enzymatic method using reagent from BIO Merieux (France) by Hitachi 705 chemistry analyzer (Japan)
3. **Serum Creatinine:** The assays were done by calometric method using reagents from Boehringer Mannheim (Germany) by Hitachi 705 chemistry analyzer (Japan)

3.6 Definition

3.6.1 Diabetic Nephropathy

Defined as a clinical syndrome that is characterized by persistent protenuria (microalbuminuria or macroalbuminuria), blood pressure elevation, with or without renal impairment, in the absence of other primary kidney disease. (Parving 2001)

3.6.2 Type 2 Diabetes Mellitus

The following criterias were used for the diagnosis of diabetes.

- Random plasma glucose > 11.1 mmol/l on 2 separate occasions with symptoms i.e. polyuria, polydipsia and unexplained weight loss or
- Fasting plasma glucose > 7.0 mmol/l on 2 separate occasions or

- 2-hour plasma glucose > 11.1 mmol/l during oral glucose tolerance test (OGTT) on 2 separate occasions

(Expert committee on the Diagnosis and Classification of Diabetes Mellitus. 1997)

3.6.3 Arterial Hypertension

Was defined as systolic blood pressure ≥ 140mmHg or diastolic blood pressure ≥ 90mmHg, or both, or patient was on antihypertensive.

(Joint National Committee. 1997)

3.6.4 Smoking Habit

Current smokers:	Subjects smoke one or more cigarette, cigar or pipe.
Ex smokers:	Defined as subjects who reported having stopped smoking
Non smoker:	Patients who never smoked

3.6.5 Hypercholesterolemia

Total Cholesterol of more than or equal to 5.2 mmol/L.

(Second Consensus Statement on Management of Hyperlipidaemia, MOH. 1998)